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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : DONG, Zheng Xin Serial No. : 10/582,534 Filed : June 9, 2006	Examiner : LUKTON, David Art Unit : 1654
Title : ANALOGUES OF GLP-1	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
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DECLARATION OF JOHN E. TAYLOR UNDER 37 C.F.R. §1.132

I, John E. Taylor, Ph.D., hereby declare and state that:

1. I have a Ph.D. in Pharmacology and I serve as Associate Director of Receptor and Cellular Biology at Biomeasure, Incorporated (hereinafter "Biomeasure"), 27 Maple Street, Milford, MA 01757-3650. It is a routine part of my job to test the receptor binding activity of novel compounds using the assay method(s) commonly employed in the field.

2. I am familiar with the subject matter claimed in the above-identified patent application, U.S. Serial No. 10/582,534

3. I understand that the last Office Action issued in this application was dated May 20, 2009, and that the Examiner of this application is of the view and stated in the Office Action that in the absence of experimental data supporting the asserted activity of the claimed compounds, Claims 1-5 and 11 contain subject matter which is not described in the specification in such a way as to enable one skilled in the art to which it pertains or with which it is most nearly connected, to make and/or use the invention.

4. I make this declaration to show that the data generated by following the procedures disclosed in this application (at page 26), which is also

described in paragraph 5 below, provide sufficient and convincing evidence that the claimed compounds of this application are specific for the GLP-1 receptors and possess the ability to evoke a GLP-1-like response from cells expressing GLP-1 receptors. One of skill in the art would readily appreciate that the efficacy of any of the compounds of the invention can be determined by using such standard assays. Thus, a person of skill in the art would have been able to determine the suitability of the compounds of Claims 1-5 and 11.

5. My colleagues and I have tested the compounds in the below Table 1 for activity as a GLP-1 binding compound according to the following procedure.

Cell Culture:

RIN 5F rat insulinoma cells (ATCC-# CRL-2058, American Type Culture Collection, Manassas, VA), expressing the GLP-1 receptor, were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, and maintained at about 37 °C in a humidifed atmosphere of 5% CO_2/95% air.

Radioligand Binding:

Membranes were prepared for radioligand binding studies by homogenization of the RIN cells in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkman Polytron (Westbury, NY) (setting 6, 15 sec). The homogenates were washed twice by centrifugation (39,000 g / 10 min), and the final pellets were resuspended in 50 mM Tris-HCl, containing 2.5 mM $MgCl_2$, 0.1 mg/ml bacitracin (Sigma Chemical, St. Louis, MO), and 0.1% BSA. For assay, aliquots (0.4 ml) were incubated with 0.05 nM (^{125}I)GLP-1(7-36) (~2200 Ci/mmol, New England Nuclear, Boston, MA), with and without 0.05 ml of unlabeled competing test peptides. After a 100 min incubation (25 °C), the bound (^{125}I)GLP-1(7-36) was separated from the free by rapid filtration through GF/C filters (Brandel, Gaithersburg, MD), which had been previously soaked in 0.5% polyethyleneimine. The filters were then washed three times with 5 ml aliquots of ice-cold 50 mM Tris-HCl, and the bound radioactivity trapped on the filters was counted by gamma spectrometry (Wallac LKB, Gaithersburg, MD). Specific binding was defined as the total (^{125}I)GLP-1(7-36) bound minus that bound in the presence of 1000 nM GLP1(7-36) (Bachem, Torrence, CA).

6. The results of the GLP-1 receptor binding assay for said compounds are shown below as Table 1:

TABLE 1

Example No.	Structures	hGLP Ki (nM)
2	(Aib^{8,35}, Arg^{26,34}, Phe^{31}, Pro^{37}, Ser^{38,39})hGLP-1(7-39)-NH_2	0.623
3	(Aib^{8,35,37}, Arg^{26,34}, Phe^{31}, Asn^{38})hGLP-1(7-38)-NH_2	0.878
10	(Aib ^{8,35,37}, Arg ^{26,34}, Phe^{31}, Ser ^{38})hGLP-1(7-38)NH_2	0.645
11	(Aib ^{8,35,37}, Gaba^{38})hGLP-1(7-38)NH_2	0.540
12	(Aib ^{8,35,37}, Arg ^{26,34}, Phe^{31}, His^{38})hGLP-1(7-38)NH_2	0.830
13	(Aib ^{8,35}, Arg ^{26,34}, Phe^{31}, β-Ala^{37}, His^{38})hGLP-1(7-38)NH_2	1.563
14	(Aib ^{8,35,37,} Arg ^{26,34}, D-His^{38})hGLP-1(7-38)NH_2	2.000
15	(Aib ^{8,35,37,} β-Ala ^{38})hGLP-1(7-38)NH_2	0.870
20	(Aib ^{8,35}, Arg ^{26,34}, β-Ala^{37}, His^{38})hGLP-1(7-38)NH_2	1.060
21	(Aib ^{8,35,37}, Arg ^{26,34}, Phe^{31}, Gly^{38})hGLP-1(7-38)NH_2	0.590
22	(Aib ^{8,35,37}, Arg ^{26,34}, Gly^{38})hGLP-1(7-38)NH_2	0.730
23	(Aib ^{8,35,37}, Arg ^{26,34}, β-Ala^{38})hGLP-1(7-38)NH_2	0.990
24	(Aib ^{8,35,37}, Arg ^{26,34}, Gaba^{38})hGLP-1(7-38)NH_2	1.290
25	(Aib ^{8,35,37}, Arg^{34}, Phe^{31}, His^{38})hGLP-1(7-38)NH_2	3.045
26	(Aib ^{8,35,37}, Arg ^{26,34}, His^{38})hGLP-1(7-38)NH_2	2.120
27	(Aib ^{8,35,37}, Arg ^{26,34}, Phe^{31}, Gaba^{38})hGLP-1(7-38)NH_2	1.890
28	(Aib ^{8,35,37}, Arg ^{26,34}, Phe^{31}, Ava^{38})hGLP-1(7-38)NH_2	1.825
29	(Aib ^{8,35,37}, Arg ^{26,34}, Ava^{38})hGLP-1(7-38)NH_2	2.710
30	(Aib ^{8,35,37}, Arg^{34}, Phe^{31}, D-His^{38})hGLP-1(7-38)NH_2	3.195
31	(Aib ^{8,35,37}, Arg^{34}, Phe^{31}, Gly^{38})hGLP-1(7-38)NH_2	2.580
33	(Aib ^{8,35,37}, Gly^{38})hGLP-1(7-38)NH_2	2.655
34	(Aib ^{8,35,37}, Arg ^{26,34}, Phe^{31}, D-His^{38})hGLP-1(7-38)NH_2	3.865

35	(Aib ^{8,35}, Arg ^{26,34}, Phe^{31}, β-Ala^{37}, D-His^{38})hGLP-1(7-38)NH_2	4.730
36	(Aib ^{8,35,37}, Arg ^{26,34}, Phe^{31}, β–Ala^{38})hGLP-1(7-38)NH_2	3.750
37	(Aib ^{8,35} , Arg ^{26,34}, Phe^{31}, β-Ala ^{37,38})hGLP-1(7-38)NH_2	4.810
38	(Aib ^{8,35,37}, Arg^{34}, Phe^{31}, β-Ala^{38})hGLP-1(7-38)NH_2	0.938
39	(Aib ^{8,35,37}, Arg^{34}, Phe^{31}, Gaba^{38})hGLP-1(7-38)NH_2	0.715

7. The results of the radioligand binding assay described herein demonstrate that the representative compounds of the present invention bind to the GLP-1 receptor with substantially the same affinity as hGLP-1(7-36)NH_2. Thus, the application supplies sufficient information to practice the invention of the claims. In view of the data presented above and the Applicant's comments, it is believed that the Examiner's concern has been addressed.

8. I declare that all statements made herein of my own knowledge are true and that statements made upon information and belief are believed to be true, and further that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: 11/18/09

John E. Taylor, Ph.D.